to volume, and mix. Use this solution to determine the resolution requirement for the chromatographic system.

(vi) *System suitability requirements*—(A) *Asymmetry factor.* The asymmetry factor (A_S), measure data point 5 percent of the peak height from the baseline, is satisfactory if it is not less than 0.85 and not more than 1.1.

(B) *Efficiency of the column.* The absolute efficiency (h_r) is satisfactory if it is not more than 10.0 for the idarubicin hydrochloride peak, equivalent to 4,500 theoretical plates for a 25-centimeter column of 6-micrometer particles.

(C) *Resolution factor.* The resolution factor (R_S) between the peak for idarubicin and 4-demethoxydaunorubicinone (generated insitu) is satisfactory if it is not less than 9.5.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation ($S_R$in percent of 5 replicate injections) is satisfactory if it is not more than 2.0 percent.

(E) *Capacity factor.* The capacity factor (k') for idarubicin hydrochloride is satisfactory if it is not less than 5 and not more than 15. If the system suitability parameters have been met, proceed as described in §436.216(b) of this chapter.

(vii) *Calculations.* Calculate the micrograms of idarubicin hydrochloride per milligram of sample as follows:

$$\text{Micrograms of idarubicin hydrochloride per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_U=Area of the idarubicin hydrochloride peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S=Area of the idarubicin hydrochloride peak in the chromatogram of the idarubicin hydrochloride working standard;

P_S=Idarubicin hydrochloride activity in the idarubicin hydrochloride working standard solution in micrograms per milliliter;

C_U=Milligrams of idarubicin hydrochloride sample per milliliter of sample solution;

m=Percent moisture content of the sample.

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 5 milligrams per milliliter.

(4) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

(5) *HPLC impurities.* Proceed as directed in paragraph (b)(1) of this section. Calculate the percentage of impurities as follows:

$$\text{Percent individual impurity} = \frac{A_i \times 100}{A_t}$$

$$\text{Percent total HPLC impurities} = \frac{A \times 100}{A_t}$$

where:

A_i=Area of the individual impurity peak;

A=The sum of areas of all peaks minus the area due to the idarubicin hydrochloride peak and solvent peak; and

A_t=The sum of areas of all peaks in the chromatogram excluding the solvent peak.

(6) *Identity.* Proceed as directed in §436.211 of this chapter, using a 1.0 percent potassium bromide disc prepared as directed in §436.211(b)(1).

[58 FR 26664, May 4, 1993]

§ 450.40 Plicamycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Plicamycin is a yellow compound and is so purified and dried that:

(i) Its plicamycin content is not less than 900 micrograms of plicamycin per milligram calculated on an anhydrous basis.

(ii) Its loss on drying is not more than 8 percent.

(iii) Its pH in an aqueous solution containing 0.5 milligram per milliliter is not less than 4.5 nor more than 5.5.

(iv) Its absorptivity on the anhydrous basis at the absorption maximum of 278 millimicrons is 100±5 percent of that of the plicamycin standard similarly treated.

(v) It gives a positive result to the identity tests for plicamycin.

(vi) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter. In addition, each package shall bear on its label the statement ''Store below 10° C. (50° F.)''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for plicamycin content, loss on drying, pH, absorptivity, identity, and crystallinity.

(ii) Samples required on the batch: 2 packages, each containing not less than 100 milligrams; and 3 packages, each containing not less than 50 milligrams.

(b) *Tests and methods of assay.* Plicamycin is more toxic than the average drug and must be handled with care in the laboratory. Avoid inhaling fine particles of powder. If the substance contacts the skin, wash with soap and water. Solutions should not be pipetted by mouth. Plicamycin is hygroscopic and care should be exercised during storage and weighing samples. Samples should be stored at 10° C. or less in a sealed, light-resistant container with a desiccant. Dispose of all waste material by dilution with larger volumes of trisodium phosphate solution.

(1) *Plicamycin content.* Proceed as directed in § 436.341 of this chapter, preparing the sample and calculating the plicamycin content as follows:

(i) *Preparation of sample solution.* Place approximately 5 milligrams of the sample, accurately weighed, into a 50-milliliter, amber volumetric flask and dilute to volume with mobile phase and mix.

(ii) *Calculations.* Calculate the micrograms of plicamycin per milligram of sample as follows:

$$\text{Micrograms of plicamycin per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u = Area of the plicamycin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s = Area of the plicamycin peak in the chromatogram of the plicamycin working standard;

P_s = Plicamycin activity in the plicamycin working standard solution in micrograms per milliliter;

C_u = Milligrams of sample per milliliter of sample solution; and

m = Percent moisture content of the sample.

(2) *Loss on drying.* Proceed as directed in § 436.200(g) of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 0.5 milligram of plicamycin per milliliter. Allow the solution to remain in contact with the electrodes until a steady reading is obtained or for 5 minutes.

(4) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 10 milligrams each of the sample and standard (dried as described in § 436.200(g) of this chapter), accurately weighed, in 50 milliliters of absolute methanol. Transfer 5-milliliter portions into 100-milliliter volumetric flasks and dilute to volume with 0.01*N* hydrochloric acid in methanol prepared by diluting 20 milliliters of 0.5*N* aqueous hydrochloric acid to 1 liter with absolute methanol. Using a suitable spectrophotometer and 0.01*N* hydrochloric acid in methanol as the blank, scan the absorption spectrum between the wavelengths of 220 millimicrons and 400 millimicrons. Determine the absorbance of each solution at the absorption maximum near 278 millimicrons. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculation:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample solution} \times \text{milligrams of standard} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard solution} \times \text{milligrams of sample} \times 10}$$

(5) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the plicamycin working standard.

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19145, May 30, 1974, as amended at 49 FR 5097, Feb. 10, 1984; 49 FR 24018, June 11, 1984]

§ 450.45 Mitomycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Mitomycin is 7-amino-9a-methoxymitosane. It is a blue-violet compound that is soluble in water, methanol, acetone, butyl acetate, and cyclohexanone. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) [Reserved]

(iii) Its moisture content is not more than 5 percent.

(iv) Its pH in a solution containing 5 milligrams per milliliter is not less than 6.0 and not more than 8.0.

(v) When calculated on the anhydrous basis, its absorptivity at 357 nanometers is not less than 95 percent and not more than 105 percent of that of the mitomycin working standard similarly treated.

(vi) It gives a positive identity test for mitomycin.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(ii) Samples required: Five packages, each containing approximately 100 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 microgram of mitomycin per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 5 milligrams per milliliter.

(5) *Absorptivity.* Determine the absorbance of the sample and standard solution in the following manner: Place an accurately weighed portion of approximately 25 milligrams of mitomycin into a 50-milliliter volumetric flask. Dissolve and dilute to volume with absolute methanol. Further dilute an aliquot with absolute methanol to 0.005 milligram of mitomycin per milliliter. Using a suitable spectrophotometer equipped with a 1-centimeter quartz cell and absolute methanol as the blank, determine the absorbance of the sample and standard solutions at 357 nanometers. Calculate the percent relative absorptivity as follows:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{percent mitomycin content of standard} \times 100}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times (100 - m)}$$

where:

m=percent moisture in the sample.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section.